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ANTHER CULTURE OF PEPPER (*Capsicum annuum L.*): THE EFFECT OF NUTRIENT MEDIA

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Introduction: The development of valuable haploid plants useful in genetics as well as in practical selection is purpose of the *in vitro* cultivation of anthers. The first data for obtained haploids via anther culture of pepper (*Capsicum annuum L.*) were published by Wang *et al.* (1973), George *et* Narayanaswamy (1973) and Kuo *et al.* (1973). The successful embryoid induction and microspore regeneration depends on a number of factors: stage of microspore development, low temperature pretreatment (Sibi *et al.*, 1979), elevated temperature treatments (Dumas de Vaulx *et al.*, 1981), genotype and donor plant age (Morrison *et al.*, 1986; Gomez & Chambonnet, 1992; Kristiansen & Andersen, 1993; Qin & Rotino, 1993; Ltifi & Wenzel, 1994; Mytiko *et al.*, 1995), culture medium (Sibi *et al.*, 1979; Vagera & Havranek, 1985; Pundeva *et al.*, 1990; Boyaci, 2001; Ellialtioglu *et al.*, 2001) etc.
The aim of the present study was to investigate *in vitro* response of anther culture of Bulgarian pepper lines, varieties and hybrids cultivated on different nutrient media.

Materials and methods: Donor plants of four lines (№№145, 146, 1312 and 1924), five varieties (Zlaten medal, Hebur, Stryama, Albena and Kourtovska kapiya) and two F1 hybrids (№1647 x №1969 and №1647 x №1962) developed in the Maritsa Vegetable Crops Research Institute-Plovdiv, were grown in greenhouse. Flower buds were collected at the late uninucleate microspore stage (Sibi et al., 1979). The excised anthers were cultivated on the following media:

1. Medium C + 2 mg/l kinetin + 0,1 mg/l 2,4-D (Dumas de Vaulx *et al.*, 1981);
2. Medium C (Dumas de Vaulx *et al.*, 1981), without growth regulators;
3. Medium C (Dumas de Vaulx *et al.*, 1981) + 0,01 mg/l kinetin and 2,4-D, respectively (Gyulai *et al.*, 2000);
4. Medium Cm + 2 mg/l kinetin and 2,4-D, resp. (Sibi *et al.*, 1979);
5. Medium Cm (Sibi *et al.*, 1979), without growth regulators;
6. Medium Cm (Sibi *et al.*,1979) + 0,01 mg/l kinetin and 2,4-D, resp.;
7. Medium MS (Murashige *et* Skoog, 1962), without growth regulators;
8. Medium MS (Murashige *et* Skoog, 1962) + 0,2 mg/l kinetin + 2 mg/l IAA;
9. Medium MS (Murashige *et* Skoog, 1962) + 2 mg/l NAA and BA, resp. (Park *et al.*, 1992);
10. Medium MS (Murashige *et* Skoog, 1962) + 0,1 mg/l kinetin + 0,004 mg/l 2,4-D (Matsubara *et al.*, 1998);

The incubation were according to Dumas de Vaulx *et al.* (1981).The frequency of anthers producing callus or direct embryoids from each genotype were recorded. The embryoids developed into microplants were presented in percentage both to the total number of anthers and to the number of obtained direct embryoids from the respective genotype.

Results and discussion: The responsive anthers from the studied genotypes reacted with callusogenesis without regeneration or with direct embryoid formation on the different tested media. There was considerable variation in the response of studied genotypes to different medium. Only in one case we registered explants with indirect organogenesis but without developed structures (medium 1). Direct embryoids developed in microplants were observed in three media (1,2,10).
The results of the effect of C media (1,2,3) are shown in Table 1. The highest embryogenic response were observed on medium 1 compared to media 2 and 3. In the same medium we observed regenerative structure formation via callus (var. Zlaten Medal) and direct embryoids developed into microplants (var. Stryama – 20% of embryos). Direct embryoid formation on medium 2 without growth regulators were observed in Stryama genotype only where 50% of embryos developed into regenerants. No embryos have been obtained from all genotypes on medium 3; there was callusogenesis only. The most of studied genotypes were unresponsive on media 2 and 3.

The results of the Cm media effect (4,5,6) are presented in Table 2. Embryogenic response were observed in medium 4 only in the most of studied genotypes (7 from the studied 10).
On one of the tested MS media (7,8,9,10) we observed the highest number of genotypes reacted with direct embryogenesis (medium 10) compared with 7, 8 and 9 media (Table 3). On the same medium we observed embryoids developed into microplants (line №145 – 33,34%).
In the media without growth regulators (2, 5, 7 – Table 1, 2 and 3) higher embryogenic response was observed on medium 7 (30% of tested genotypes) but without development to regenerants. No embryos have been obtained in medium 5. Direct embryo formation on medium 2 was shown only in Stryama genotype and 50% of embryoids develpoed to microplants. From this we can conclude that the lack and also the lower content of tested plant growth regulators (kinetin and 2,4-D) in the media don't provoke the direct embryogenesis but often influence positively callusogenesis.
From all the tested media (Tabl. 1, 2 and 3) the highest number of genotypes (72,7% of all tested) with direct embryo formation but without development into regenerants we registered on medium 4. Probably the higher auxin – cytokinin ratio influences positively on this process. The embryogenic response observed in media 1 and 10 (54,5% and 55,6% of all tested genotypes resp.) results in microplants formation (20% and 33,34% of embryoids, resp.). It is very probably the regeneration process in this case is in the result of suitable auxin – cytokinin ratio (lower auxin concentration : higher cytokinin concentration) in the media.
In all of the studied media and in almost all of the studied genotypes was registered comparatively low level of regenerative activity.The considerable variation in the response of different genotypes to different media suggest evident genotypic "preferences".

Conclusions:

- The *in vitro* response of studied pepper anther culture to a great extent depends on donor plant genotype, medium composition, supplements and growth regulators.
- The media C and MS with lower auxin concentration to the cytokinins are suitable for direct embryogenesis in anther culture of the most of the tested genotypes.

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Tab.1. Callusogenesis, embryogenesis and regeneration on variants of C media (in %).

media	1						2					3				
	reacted (%)			regenerants (%)			reacted (%)			regenerants (%)		reacted (%)			reacted (%)	
genotypes	total	direct embryoids	callus	indirect organogen.	to number of anthers	to direct embryoids	total	direct embryoids	callus	to number of anthers	to direct embryoids	total	direct embryoids	callus	to number of anthers	to direct embryoids
N 145	12,07	0	12,07	0	0	0	-	-	-	-	-	9,09	0	9,09	0	0
N 146	10,2	0	10,2	0	0	0	0	0	0	0	0	8,82	0	8,82	0	0
N 1312	1,28	1,28	0	0	0	0	2,78	0	2,78	0	0	-	-	-	-	-
N 1924	27,45	0	27,45	0	0	0	0	0	0	0	0	0	0	0	0	0
Zl. Medal	44,45	4,94	38,27	1,23	0	0	0	0	0	0	0	0	0	0	0	0
Hebur	21,15	7,69	13,46	0	0	0	2,44	0	2,44	0	0	0	0	0	0	0
Stryama	26,09	10,87	15,22	0	2,17	20	6,67	1,91	4,76	0,95	50	0	0	0	0	0
Albena	36,99	10,96	26,03	0	0	0	-	-	-	-	-	61,12	0	61,12	0	0
K. kapyja	9,76	0	9,76	0	0	0	-	-	-	-	-	0	0	0	0	0
F1 1647x1969	2,7	0,68	2,03	0	0	0	0	0	0	0	0	13,43	0	13,43	0	0
F1 1647x1962	15,18	0	15,18	0	0	0	-	-	-	-	-	0	0	0	0	0

Tab. 2. Callusogenesis, embryogenesis and regeneration on variants of Cm media (%).

media	4				5				6			
	reacted (%)			regenerants	reacted (%)			regenerants	reacted (%)			regenerants
genotypes	total	direct embryoids	callus	to direct embryo (%)	total	direct embryoids	callus	to direct embryo (%)	total	direct embryoids	callus	to number of embr.(%)
N 145	47,83	2,17	45,65	0	37,5	0	37,5	0	14,81	0	14,81	0
N 146	20,84	0	20,84	0	8,89	0	8,89	0	-	-	-	-
N 1312	14,55	1,82	12,73	0	-	-	-	-	11,76	0	11,76	0
N 1924	5,17	0	5,17	0	-	-	-	-	-	-	-	-
Zl. Medal	3,45	3,45	0	0	6,25	0	6,25	0	9,09	0	9,09	0
Hebur	10,35	3,45	6,9	0	-	-	-	-	-	-	-	-
Stryama	22,23	5,56	16,67	0	3,7	0	3,7	0	-	-	-	-
Albena	33,93	7,14	26,79	0	0	0	0	0	-	-	-	-
K. kapyja	0	0	0	0	-	-	-	-	-	-	-	-
F1 1647x1969	26,55	0,89	25,66	0	0	0	0	0	7,69	0	7,69	0
F1 1647x1962	19,28	4,82	14,46	0	0	0	0	0	11,32	0	11,32	0

Tab. 3. Callusogenesis, embryogenesis and regeneration on variants of MS media (%).

media	7				8				9				10				
	reacted (%)			regenerants	reacted (%)			regenerants	reacted (%)			regenerants	reacted (%)			regenerants (%)	
genotypes	total	direct embryoids	callus	to number of embr.(%)	total	direct embryoids	callus	to number of embr.(%)	total	direct embryoids	callus	to number of embr.(%)	total	direct embryoids	callus	to number of anthers	to number of embryoids
N 145	0	0	0	0	9,62	0	9,62	0	21,85	1,68	20,17	0	13,51	8,11	5,41	2,7	33,34
N 146	13,6	0	13,6	0	38,1	0	38,1	0	30,19	0	30,19	0	10,53	0	10,53	0	0
N 1312	0	0	0	0	5,56	0	5,56	0	10	0	10	0	7,69	7,69	0	0	0
N 1924	0	0	0	0	3,7	0	3,7	0	-	-	-	-	0	0	0	0	0
Zl.medal	-	-	-	-	12,3	0	12,28	0	-	-	-	-	18,57	5,71	12,86	0	0
Hebur	0	0	0	0	-	-	-	-	0	0	0	0	8,34	0	8,34	0	0
Stryama	5,26	0	5,26	0	22,2	0	22,23	0	16,67	0	16,67	0	10	4	6	0	0
Albena	6,67	1,12	5,56	0	6,67	0	6,67	0	38,71	9,68	29,03	0	-	-	-	-	-
K.kapyja	0	0	0	0	17,7	0	17,65	0	0	0	0	0	-	-	-	-	-
1647x1969	18,3	2,82	15,5	0	17	0	17,02	0	38,89	0	38,89	0	0	0	0	0	0
1647x1969	53,9	11,54	42,3	0	5,79	0,83	4,96	0	1,41	0	1,41	0	44,45	11,12	33,34	0	0